

“Black-Light” Induction of Photoperiod-controlled Diapause Responses of the Viceroy Butterfly, *Limenitis archippus* (Nymphalidae)

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Abstract. The nearctic viceroy butterfly, *Limenitis archippus* (Cramer), is a typical long-day insect. The 2nd and early 3rd instars are photosensitive. Half-grown larvae respond to short-day (autumn) photoperiod by constructing hibernacula and entering diapause. Long-day photoperiod induces rapid growth and direct development. Previous experiments testing this facultative response have used fluorescent bulbs emitting both UV and visible light (320-700 nm). However, both diapause and direct development can be induced in larvae exposed to “black-light” photoperiod regimes containing mainly the near UV (violet) and UV portions of the spectrum (320-436 nm). The stemmata and dorsolateral abdominal saddlepatch areas of the 2nd and 3rd instar larvae represent possible photoreceptors for mediating these responses.

Introduction

Many temperate insects respond to changes in daylength through hormonally regulated physiological mechanisms influencing direct development or diapause (Lees, 1955, 1960; Danilevskii, 1965; Andrewartha & Birch, 1973, Saunders, 1976, 1977; Tauber & Tauber, 1973, 1976; Beck, 1980). Typical long-day insects exhibit diapause when exposed to short daylength (< 11 hr light per 24 hr day) but show direct development under longer photoperiods. Such environmentally induced responses are termed facultative. The photosensitive stages vary between species, and may involve eggs, larvae, pupae, or adults (Wigglesworth, 1970, 1972). Such responses exhibit variability within and between broods in a single species. Temperate insect strains from different latitudes or altitudes, exhibit similar variation. (Tauber & Tauber, 1972; Beck, 1980; Ujiye, 1985). Diapause is related to insect endocrine control and metabolism (Gilbert et al, 1960; Harvey, 1962; de

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Wilde, 1965; Harvey and Haskell, 1966), and is under polygenic control in most species. Recently, a dual system involving photoperiod and temperature for regulating circadian rhythms and diapause has been proposed (Beck, 1977; Neumann & Krüger, 1985).

Facultative diapause in the nymphaline butterfly *Limenitis (Basilarchia) archippus* (Cramer) has been studied by Clark & Platt (1969), Hong & Platt (1975), Frankos & Platt (1976), and Williams & Platt (1987). This species enters larval diapause when exposed to short-day photoregimes at room temperature. Second and early 3rd instar larvae respond to photoperiod over a seven to ten day period. Larvae preparing to diapause grow more slowly than their long-day siblings. Those reared in either continuous light or in complete darkness exhibit nearly 100% mortality (Platt, pers. obs.). Diapause initiation and termination take place while development is arrested in the 3rd instar. Thus, the diapause responses of *Limenitis* are not complicated by developmental processes associated with metamorphosis, as they are in other species which diapause as pupae.

Complex innate behavior precedes larval diapause and can be conveniently studied in short-day larvae. This behavior is genetically based (Clark & Platt, 1969; Hong & Platt, 1975), but is expressed only under short-day conditions. Short-day larvae grow slowly and construct hibernacula (overwintering chambers) from the basal portions of poplar (*Populus spp.*) and willow (*Salix spp.*) leaves (*Salicaceae*) on which they feed. These tubular structures are made by chewing the leaf in a characteristic manner, and then covering the remaining basal leaf surfaces with silk to form an enclosed curving tube which remains open at its outer (distal) end. The hibernaculum is attached to the foodplant twig with a substantial silk girdle formed around the leaf stem and surrounding next season's bud (Edwards, 1884; Scudder, 1889; Weed, 1926; Klots, 1951). The hibernaculum remains attached to the plant throughout the winter months following leaf drop. Short-day larvae crawl into these chambers prior to diapausing. Physiological changes such as water loss and glycerol accumulation accompany diapause (Frankos & Platt, 1976). Diapause onset can be reversed by switching short-day larvae to long-day conditions (LD 16:8), even after hibernaculum formation and entry have occurred. However, the reciprocal transfer of 2nd and 3rd instar larvae (from long-day to short-day photoregimes) usually results in larval death (Clark & Platt, 1969).

Few papers have addressed the wavelengths (colors) of light which influence insect diapause. Beck (1980) states that the most effective wavelengths are between 400-550 nm for most species. Bünning and Joerrens (1960) found that blue light induced diapause in *Pieris*

²Larvae were not reared in photochambers containing the insect "black-light" bulbs, because plants placed in these chambers wilted within 12 hrs, and their leaves dried up. This evidently resulted from the light quality emitted by these particular bulbs.

brassicae L. (Pieridae) during early photophase, but that red light promoted diapause later in the diel photoperiod cycle. However, Beck (1980) criticized their conclusions, which he believed could be explained more simply by assuming that the red light regime was equivalent to total darkness in their experiments. The spectral limits for diapause induction in *Limenitis* have not been previously determined.

In this paper we show that *Limenitis* larvae exhibit normal diapause responses when grown in photochambers containing fluorescent "black-light" bulbs emitting a partial spectrum of violet-blue and ultraviolet (UV) wavelengths. UV light is known to be an important spectral component of many insects, especially Lepidoptera (Silbergleid, 1979). Clark & Platt (1969) suggested that the dorsolateral grey-white abdominal saddlepatch of young larvae may represent a photosensitive region. This pale saddlepatch makes the small larvae cryptically patterned, and helps them resemble bird droppings when at rest in curved positions on the leaves and twigs of their foodplants. The stemmata (ocelli) of larvae also are known to possess UV receptors (Ichikawa & Tatda, 1982).

Materials and methods

All larvae were reared from eggs in light-tight wooden photochambers (61 × 61 × 42 cm inside dimensions) at room temperature (25 ± 2°C). The inside walls of the chambers were painted a non-fluorescent flat white. Experimental chambers contained single 15" G. E. fluorescent "black-light" bulbs emitting a partial spectrum between 320-436 nm [so-called "poster lights" Fig. 1A]. The quality of light emitted by these bulbs is a deep, dim violet. The "poster light" bulbs are dark purple in color, but the wavelengths emitted are not the same as those of "black-lights" used commonly to attract and trap insects (Fig. 1B)². Control chambers contained G. E. cool white fluorescent bulbs identical in size and wattage, which emitted wavelengths throughout the visible spectrum and some UV as well (Fig. 1C).

The emission spectra were measured as follows: Light from each type of source passed through an Oriel computer-driven monochromator to a photomultiplier (PM) tube (Hamamatsu R-928) through an evacuated quartz window. The lamp's output was measured at each nm from 300-600 nm by averaging the digitized photocurrent of the PM tube (200 measurements per nm), without correction for the spectral response curve of the PM tube. These data were subsequently normalized to the peak value. The different emission spectra obtained from the three kinds of fluorescent tubes are shown for comparison in Fig. 1.

The larvae were reared on rooted cuttings of weeping willow (*Salix babylonica* L.) in moist conditions using long-day (LD 16:8) and short-day (LD 8:16) photoperiods. The onset of photophase occurred at 8:00 a.m. EST in all chambers. A total of 81 larvae was reared during the experiments.³

Individual larvae were taken from stock cultures maintained in the laboratory, or were collected as diapausing larvae in hibernacula. Eggs from three *L. archippus* females, which had been hand-paired using Platt's (1969) method, were used in the induction experiments (Table 1). Eggs from each brood were

divided evenly between the control and experimental conditions. Diapause termination studies were done using overwintering *L. archippus* larvae obtained by collecting hibernacula from the vicinity of Conowingo Dam, Cecil Co., Maryland (Table 2). Some wild-collected larvae had been parasitized by wasps, as noted in the table.

Photochambers were checked daily to determine the condition of the eggs, developing larvae, and the foodplants. Plants and larvae were misted with water each time they were examined. The laboratory in which the photochambers were kept was light-tight, and was totally darkened before the chambers themselves were opened. Under these conditions the "black-lights" emitted so little visible light that it was difficult to find the individual eggs and larvae in the experimental chambers.

Results

Results of the diapause induction experiments are shown in Table 1. The experimental "black-light" and control white light chambers gave comparable overall results. All surviving larvae on long-day underwent direct development whereas the majority of short-day larvae entered diapause. Only four short-day larvae showed direct development under "black-light". None of the short-day insects reared in white light developed directly. Mortality was minimal (11.5%) for the early larval instars and was evenly distributed among the groups.

Chi square analyses of the Table 1 data show no significant differences exist between the "black-light" and control regimes when comparing the numbers of larvae which died, developed directly, or those which diapaused. For short-day, a 2×3 contingency test yielded $\chi^2 = 4.43$, with $p > 0.10$. For long-day, a 2×2 analysis gave $\chi^2 = 0.31$, with $p > 0.05$. Thus, the null hypothesis that the larval responses are the same in both the experimental and control photoregimes is upheld in both cases.

All of the diapause termination experiments (Table 2) were carried out using long-day photoperiods. Resumption of larval activity following diapause is independent of photoperiod in *L. archippus*. Repeated observations by Platt (unpubl.) show that diapause termination can take place either in long-day or short-day photoperiods. Our present experiments show that diapause termination occurs equally well in

³In a preliminary experiment to determine whether larvae would survive in the "black-light" photochambers, four eggs of the banded purple butterfly, *Limenitis arthemis* (Drury), from Vermont were placed on a willow plant in experimental ("black-light") conditions. One larva soon disappeared, and could not be found in the dim light. The other three grew well, underwent normal metamorphosis, and produced perfect adults. Both *L. arthemis* and *L. archippus* exhibit similar facultative diapause responses (Greenfield & Platt, 1974). The availability of strains was the sole criterion for choosing which species to use in the main experiments.

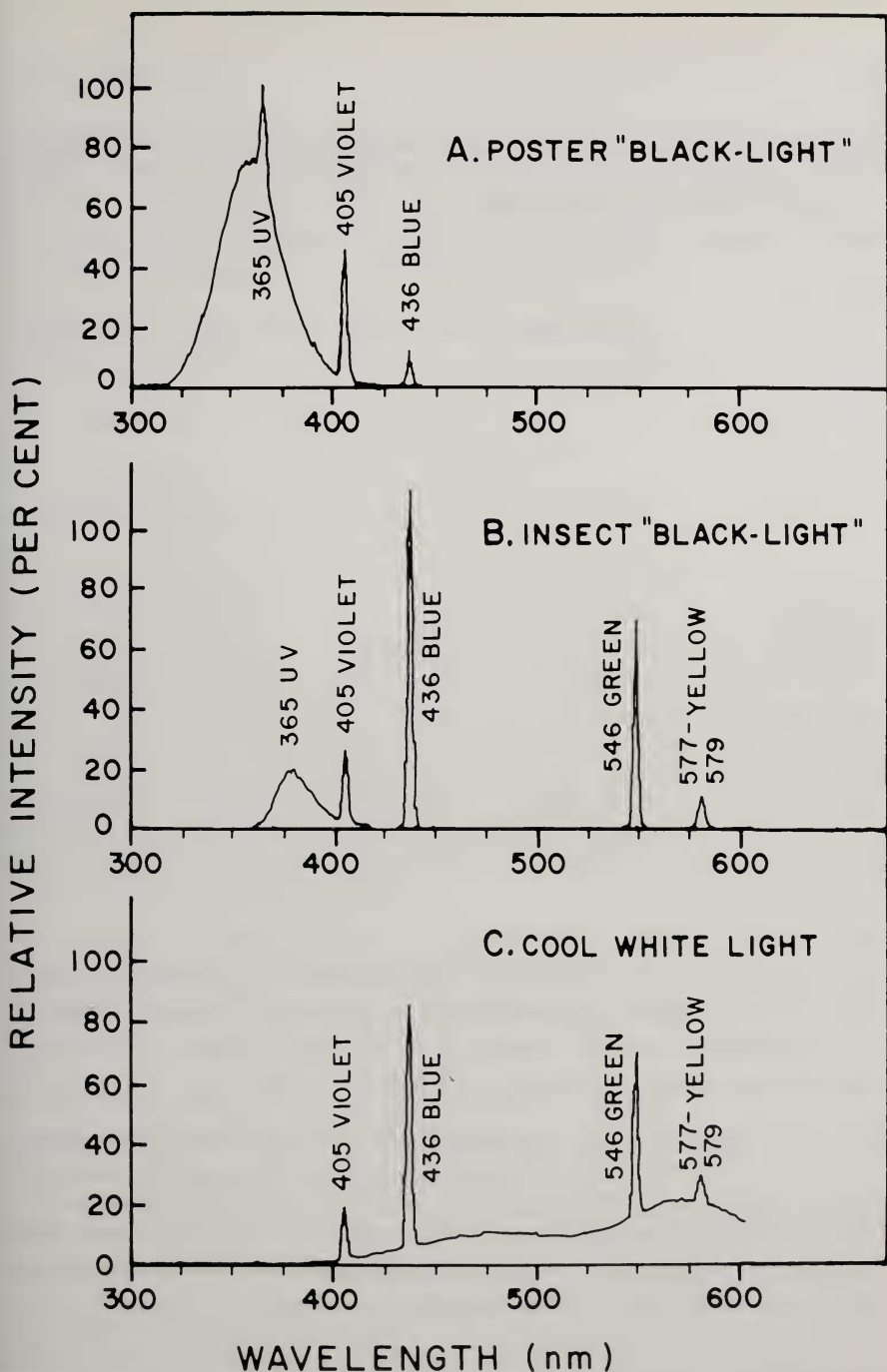


Fig. 1. Comparative emission spectra of G. E. fluorescent bulbs (No. F15T8, 15 watt). The poster "black-light" (A) emits a partial spectrum with most wavelengths within the UV range (between 313-400 nm), and peaking at 365 nm. Additional visible lines occur at 405 nm and 436 nm, producing a dim, deep violet color. Both the insect "black-light" (B — shown for comparative purposes only), and the cool white tube (C) have additional emission peaks at 546 nm and between 577-579 nm as well. These spectra were measured for us by T. W. Cronin of UMBC using a microspectrophotometer as described in the methods section.

Table 1. Diapause induction in Maryland *L. archippus* larvae subjected to "black-light" (experimental) and white light (control) photoperiods.

| Photophase | Short-day (LD 8:16) | | Long-day (LD 16:8) | |
|--|---------------------|-------------|--------------------|-------------|
| | "Black-light" | White light | "Black-light" | White light |
| Initial No. of eggs | 21 | 20 | 10 | 10 |
| No. & % dying | 1 (4.7%) | 2 (10%) | 3 (30%) | 1 (10%) |
| No. & % of survivors diapausing | 16 (80%) | 18 (100%) | 0 (0%) | 0 (0%) |
| No. & % of survivors showing direct development | 4 (20%) | 0 (0%) | 7 (100%) | 9 (100%) |

both the "black-light" and white light chambers under long-day conditions. Emerging larvae in both groups completed development successfully. All mortality encountered in these experiments was attributed to larval parasitism, which was equal in both test groups. A 2×2 chi square test of these data gave $\sum \times \frac{2}{1}$ (Yates) = 0.00, with $p > 0.90$. All of the larvae used in these experiments had been wild-collected. Thus, they had been exposed to ambient (outdoor) photophase until we collected them in December and January prior to the experiments. The larvae exhibited no apparent physiological or metabolic difficulties adjusting to either the artificial photoregimes or other laboratory conditions to which we subjected them.

Discussion

Insects in general, and Lepidoptera in particular possess visual sensitivity which peaks in the UV range between 320-400 nm (Lutz, 1933; Goldsmith, 1961; Ferris, 1972; Goldsmith & Bernard, 1974; Platt, et al, 1984). However, Lepidoptera and other insects can perceive colors in the visible portion of the spectrum as well (Burkhardt, 1964; Ichikawa & Tateda, 1982). Insect perception of infrared wavelengths

Table 2. Diapause termination in over-wintering *L. archippus* larvae collected in hibernacula near Conowingo Dam (Cecil Co.) MD. Larvae were reared under long-day (16L:8D) photoperiod at room temperature with high moisture in the chambers.

| | <i>Black light</i> | <i>White light</i> |
|--|--------------------|--------------------|
| No. of hibernacula with live larvae | 10 | 10 |
| No. & % dying (all parasitized) ¹ | 4 (40%) | 3 (30%) |
| No. & % of survivors maturing to adults | 6 (100%) | 7 (100%) |

¹The braconid wasp parasite was *Apanteles limenitidis* Riley. Parasitized larvae emerged from their hibernacula (terminated diapause) and fed briefly. Soon they became inactive and never molted beyond third instar. Wasp maggots emerged from the dying larvae and spun tiny yellow silk cocoons nearby. Each larva yielded a single wasp. Adult wasps emerged about one week later. The parasites obviously regulate their own cyclic development to correspond with that of their host, since the larvae had been parasitized the previous autumn prior to entering their hibernacula.

(600 nm and above) has not been well-documented in most species. Often the individual ommatidia of insect compound eyes are specialized for receiving a specific spectral range. Individual stemmata (important larval photoreceptors) also are known to respond to specific wavelengths in the same manner (Ichikawa & Tateda, 1982). However, these stemmata (larval ocelli) are not believed to be functional in diapause responses (Tanaka 1950, a, b, c, 1951, a, b).

Insects can respond to low intensities of light. Chapman (1969) states that intensities above 1.0 foot-candle (f.c.) are sufficient to induce photoresponses. Above this level, light intensity is not important in insect photoreception. In both our experimental and control photochambers the light intensities were well above these minimal levels. Earlier studies on *L. archippus* larvae done in our laboratory revealed that white light intensities as low as 0.10 f.c. were sufficient for inducing either larval diapause or direct development, although adults from larvae reared under such extreme conditions often possessed malformed wings (Frankos & Platt, pers. obs; Platt, 1984).

Our experiments demonstrate that exposure to short-day "black-light" photophase regimes induces facultative larval diapause in *L. archippus*. Photoregimes consisting of the blue-violet and UV portions

of the spectrum will either induce and terminate diapause, or produce direct development, just as well as those consisting of white light do. Only four of 20 larvae in one short-day "black-light" group failed to diapause, but even here 80% of the larvae made hibernacula and entered diapause as did all under white light. The failure of all larvae to enter diapause is not unexpected for such a complicated life history trait which is under polygenic control (Hong & Platt, 1975; Williams & Platt, 1987). Failure to diapause occurs in natural populations of *Limenitis* spp. in the late summer and autumn. As the ambient photoperiod decreases, a few prediapause larvae continue to show direct development late in the season, when most others are diapausing (Weed, 1926; Greenfield & Platt, 1974). The "black-light" portion of the spectrum may not be the only portion to which these larvae respond. This portion may not even be necessary for diapause induction, but it is sufficient for eliciting the same responses that occur in white light. Clark & Platt (1969) suggested that the grey-white saddle patch of 2nd and 3rd stage *Limenitis* larvae may represent a photosensitive region. Future experiments are planned to investigate the possibility that the saddle patch is photosensitive, as well as a cryptic pattern.

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